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ORIGINAL ARTICLE

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Multiple paternity and colony homeostasis in *Lasius niger* ants

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Abstract Multiple mating by social insect queens is a common phenomenon despite likely imposing substantial costs on queens. Mating with several males could be adaptive if a more genetically diverse worker force is better able to always handle any task sufficiently well, leading to a higher colony homeostasis. If multiple-paternity colonies are more homeostatic, then I propose that they may constitute less stressful rearing environments for developing sexuals. The effective stress levels experienced by developing males and queens may, however, also depend on colony productivity and sex-ratio preferences. I tested these hypotheses in the ant *Lasius niger* by examining whether the fluctuating asymmetry, means and coefficients of variation of a set of phenotypic traits in males and new queens co-varied with the effective number of patrilines per colony, colony productivity or sex ratio.
Little support was found that the level of intra-colonial genetic diversity affects the variation of phenotype in sexuals. In 1 out of 2 years, however, females from colonies with high effective patriline numbers were heavier relative to their head width than were females from colonies with few patrilines. Support was found for the hypothesis that colonies with more resources may invest more in individual sexuals, and tendencies suggested that sexuals may receive better treatment when they belong to the majority sex of their colony.

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Introduction

Multiple mating by females is widespread despite probably often imposing considerable mortality costs on females (Boomsma and Ratnieks 1996; Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Crozier and Fjerdingstad 2001; Simmons 2001). Substantial effort has been invested in proposing and testing hypotheses on how multiple mating may be adaptive in spite of its costs (Crozier and Page 1985; Bourke and Franks 1995; Keller and Reeve 1995; Crozier and Pamilo 1996; Arnqvist and Nilsson 2000; Jennions and Petrie 2000). In social insects, most theory and empirics have considered benefits that multiply-mated queens might obtain through the effect of increased genetic diversity on colony fitness (reviews by Crozier and Page 1985; Keller and Reeve 1994; Bourke and Franks 1995; Boomsma and Ratnieks 1996; Crozier and Pamilo 1996; Palmer and Oldroyd 2000; Crozier and Fjerdingstad 2001; Simmons 2001). Such benefits could be that multiple-paternity colonies would be more resistant to parasites and pathogens and better at dealing with infections (Hamilton 1987; Sherman et al. 1988, 1998; Schmid-Hempel 1998), and that a more genetically diverse worker force would be more efficient, leading to a higher mean colony performance (Crozier and Page 1985; Robinson and Page 1989; Bourke and Franks 1995) (evidence for genetic components of behaviour reviewed by Crozier and Fjerdingstad 2001).

As modification of the worker force efficiency hypothesis, Page et al. (1995) proposed that more genetically diverse colonies may not be better than less diverse colonies as far as concerns any one task or component of importance for colony fitness (i.e. means are the same); more diverse colonies may just be better at never failing badly in any area (i.e. have a lower variance in performance). Thus colonies with a greater genetic diversity

might always do averagely well at any given task because such colonies would likely always contain at least some workers who would respond quickly to increasing colony needs related with that task (water collection, food collection, temperature regulation etc.) (cf. Page et al. 1995). If such is the case then more genetically diverse colonies should have a lower variance in the different resource levels, temperature and disease impact. I therefore suggest that genetically diverse colonies may constitute more homeostatic (stable) environments for developing brood. Hence, developing sexual brood (males and new queens) may be subjected to less stress in multiple- than in single-paternity colonies.

The degree of genetic diversity of social-insect colonies may, however, not be the only factor affecting the developmental environment of sexuals. In colonies with a high reproductive output (many resources invested in sexuals), this high output could come at the cost of stressing workers and hence lowering the homeostasis of the nest environment (for any given level of intra-colonial genetic diversity). Also, the sex ratio of colonies (Boomsma and Grafen 1991; Bourke and Franks 1995; Crozier and Pamilo 1996; see also Hamilton 1964) could be associated with the effective nest environment experienced by developing sexuals, because the minority sex in a colony may experience less attention from workers or even harassment (cf. male-killing in ants and wasps, e.g. Aron et al. 1995; Keller et al. 1996; Passera and Aron 1996; Sundström et al. 1996; Henshaw et al. 2002; see also Boomsma and Grafen 1991; Queller and Strassmann 1998; Chapuisat and Keller 1999; Mehdiabadi et al. 2003).

If some colony types constitute more stressful environments for sexuals than others, then we may expect to find this reflected in the phenotype of mature sexuals. Based on studies on phenotypic responses to stress in other organisms (e.g. other insects), social insect sexuals from more stressful colonies would be predicted to show a smaller and more variable size (lower mean and higher coefficient of variation) (cf. for example, Imasheva et al. 1997; Clarke 1998; Hoffmann and Schiffer 1998; Woods et al. 1999; see also Hoffmann and Woods 2001). Also, the levels of fluctuating asymmetry (FA) (the random deviation in size of left and right sides of a bilateral trait), which is widely used as an estimator of developmental stability in the face of environmental stress (e.g. Palmer and Strobeck 1986; Leung and Forbes 1996; Møller and Swaddle 1998), might be lower for sexuals that grow up in more stressful colonies. That social insect sexuals do vary considerably in size also in monomorphic species has been reported in several cases (e.g. Ward 1983; Herbers 1990; Backus 1993; Fjerdingstad and Boomsma 1997; Wiernasz et al. 2001).

The hypothesis that more stressful colony environments should have more variable and/or smaller sexuals with a higher FA is not invalidated by the fact that social insect workers (who rear the sexual larvae) should try to counter environmental influences. This is because unpredictable resource shortages may make it impossible for workers to produce all sexuals of the optimal size, forcing workers to feed all developing sexuals less or vary how much food they provide to different sexuals of the same brood (or to kill some sexuals). Such resource shortages at unpredictable times would be expected to occur more frequently in colonies with a lower genetic diversity or in colonies that are stressing workers by rearing large numbers of sexuals.

In the present paper, I test my hypotheses of associations of multiple-paternity, colony productivity and colony sex ratio with colony homeostasis in the monogynous (one queen per colony) and polyandrous ant *Lasius niger* (Van der Have et al. 1988; Boomsma and Van der Have 1998; Fjerdingstad et al. 2002, 2003). I examined colony mean fluctuating asymmetry, and colony means and coefficients of variation of trait size for several morphological traits that reflected pre-pupal and post-eclosion feeding and development in mature males and (unmated) young, mature queens from natural mature colonies. The sampled colonies differed in sex ratio, productivity, and in intra-colonial genetic diversity due to variation in the number of mates per mother queen (as established through microsatellite marker analyses, Fjerdingstad et al. 2002). Multiple-paternity colonies of *L. niger* do not have a higher productivity (greater output of sexuals) (Fjerdingstad et al. 2003), but this does not invalidate the hypothesis that sexuals from such colonies experience a more stable developmental environment and that this could select for multiple mating by queens. This is because the quality of sexuals (which is likely to depend on their rearing conditions), not only the number of sexuals produced, will affect the fitness of a colony.

The literature of the last 5 years has shown that FA is not a fail-safe indicator of environmental stress but may vary with the specific traits, stresses and organisms examined (e.g. Hoffmann and Woods 2001; Hogg et al. 2001; see also Polak et al. 2002). However, I incorporated FA analyses in my study to make it as inclusive as possible.

Methods

Sampling, paternity assignment, colony productivity and sex-ratio estimation

I collected young (unmated) mature queens and males in traps as they tried to leave their natal colonies at the onset of their mating flights in 1997 and 1998 on the campus of Lausanne University in Switzerland (see Fjerdingstad et al. 2002 for further details). These sexuals had spent all of their life till then within their natal colonies, being reared and cared for by the workers. The numbers of fathers per colony were determined through DNA analysis of 20 young queens and 5 males per colony using 2 highly variable microsatellite markers (Fjerdingstad et al. 2002). Approximately 60% (35 out of 59) of the colonies contained several patrilines of young queens, revealing that the mother queen had mated multiply. The rest of the colonies had only a single father. The mean effective number of patrilines (which takes into account not only the number of fathers but also their proportional representation in the brood, cf. Boomsma and Ratnieks 1996) was 1.70±0.34 (SD) in 1997 (n=16) and 1.70±0.23 in 1998 (n=19). Throughout all tests that I present here,

the effective number of patrilines was used as estimator of each colony's intra-colonial genetic diversity.

For multiple-paternity colonies, I selected the young queens to be examined from a single patriline in each colony (the majority patriline or, in the case of equal paternity of two fathers, from a randomly chosen one), to keep the genetic variation in the examined sets of young queens at the same level in both single- and multiple-paternity colonies. The male samples were selected at random among the males from a given colony because males carry only maternal genes (males arise from unfertilised eggs in the Hymenoptera; Cook 1993). Multiple mating by queens would affect the genetic variance in the males produced by a colony if worker production of males (through arrhenotoky) occurred. Worker reproduction is, however, very rare in *L. niger* (Fjerdingstad et al. 2002) and so can be ignored here.

The productivity of each colony (dry biomass of sexuals with correction for the higher metabolic rate of males; Boomsma and Isaaks 1985) and its proportional investment in queens (sex ratio) were estimated by trapping (in tent traps) all sexuals that tried to fly out to mate during the entire (1997) or almost entire (1998) mating season (Fjerdingstad et al. 2002). Single- and multiple-paternity colonies did not differ in sex ratios (Fjerdingstad et al. 2002) or in productivity (Fjerdingstad et al. 2003).

Phenotypic traits examined

I measured the body weights and head widths of 7 young queens per colony for 22 and 31 colonies in 1997 and 1998, respectively, and 5 males per colony from 21 and 25 colonies, in the same 2 years. For males, dry weights were taken (methods in Fjerdingstad et al. 2002) but for young queens fresh weights were used because carrying out DNA analyses to assign queens to patriline would not have been possible with dried queens. Fresh and dry weights are highly correlated in young *L. niger* queens (r=0.89–0.93, E.J. Fjerdingstad, unpublished data). Measurements were taken using a Toledo-Mettler balance and a Nikon projector-screen micrometer.

Additional traits were measured for a subset of colonies/years. For males, I thus measured the length of the right hindmost tarsal leg segment, forewing length and width, fluctuating asymmetry of wings, total carbohydrate (sugar) content (i.e. the amount of flight fuel available; Passera et al. 1990) and sperm production. Carbohydrate content was assessed by analysis of male thoraces using the colorimetric methods of Van Handel (1985) and Van Handel and Day (1988). As a modification of Van Handel's (1985) method, the exoskeleton fragments were spun down before the optical density of the anthrone-sample solutions was measured. Carbohydrate content was measured for 4.7±0.52 (SD) mature males per colony for 30 colonies in 1998.

Male sperm content was assessed by dissecting out the genitalia of males, releasing the sperm into a buffer containing Hoecht's stain (which stains the nucleus), and then counting the sperm using a cell-count chamber and a microscope fitted for fluorescent microscopy (Sakaluk and O'Day 1984; Reichardt and Wheeler 1995; Fjerdingstad and Boomsma 1997). In this manner, all the sperm that a male will produce in his entire life-time can be assessed because the testes degenerate before maturity in ant species where males disperse before mating (Hölldobler and Wilson 1990), as is the case in *L. niger* (Van der Have et al. 1988; Boomsma and van der Have 1998; Fjerdingstad et al. 2002). Sperm load sizes were determined for 5 males per colony for 21 colonies in 1997.

For young queens, the fat content (in % of body weight) was measured in 1998. Such fat stores constitute a body reserve for brood production (Keller and Passera 1989). Fat content was estimated by oven-drying queens, weighing them, then boiling off the fat of each body in petroleum-ether and reweighing queens. 7.1±0.48 (SD) young queens per colony for 26 colonies were examined.

Fluctuating asymmetry of wing length and width (males) or cubital wing cell length (young queens) was estimated by taking replicate measurements on wings on separate days, or after having taken at least 100 measurements on other individuals. FA was analysed using a modified version of Palmer's (1994a) ANOVA method, with Individual as random factor, Side and Measurement-round as fixed factors, and FA as the Side×Individual interaction component. There was no significant directional asymmetry (DA) in males or queens; the FA variance components were significant (P<0.0005), with error constituting only 8–9% of FA. FA was not related with trait size (results not shown). The two estimators of FA in males (wing length FA and wing width FA) were positively associated across individual males (Spearman rank correlation r=0.26, n=120, P=0.005).

A general wing-FA estimator was created for males by adding the absolute (unsigned) wing width FA and wing length FA values for each individual after standardising by subtracting the mean and dividing by the standard deviation. The standardisation allowed both variables to count equally in the combined estimate. For queens, the average right minus left difference was used directly. FA of young queens was examined in 7 young queens per colony for 22 and 32 colonies in 1997 and 1998, respectively. For males, FA was studied for 5 individuals per colony for 21 colonies in 1997.

Statistical transformations and analyses

Because colony mean values for the different traits were in many cases inter-correlated (correlation coefficients ranging from r=0.50 to 0.70), I applied Principal Components Analyses (PCA) (based on correlation matrices of colony means or colony coefficients of variation) whenever more than one variable was involved in an analysis. PCAs were applied separately to each data set (i.e. sexes and years were not pooled). PCA allowed me to transform the inter-correlated variables into a set of orthogonal (uncorrelated) variables (principal components) (Jobson 1996). The first principal component (PC1), which explained most of the variation, was generally positively associated with all trait variables and could hence be considered an estimator of general size. PC2 (and PC3 where applicable) constituted a contrast between the original variables (e.g. PC2 would be negatively correlated with raw data variable 1 and positively with raw data variable 2, or the reverse).

For each set of analyses, I present the exact P values obtained and also indicate which of them remained significant after Bonferroni correction for multiple tests. The corrections for multiple tests were applied within each set of analyses. In my tests on mean body size, I examined two principal components (arising from body-weight and head-width data) and hence corrected my significance level to α=0.05/2=0.025. In the tests on mean energy reserves (examining fat content in young queens and carbohydrate contents in young males), I equally corrected for two tests. In the test on wing dimensions and male sperm load size, I studied three principal components and hence corrected the significance level to α=0.05/3=0.017. My tests on FA treated two traits, wing dimension FA and wing cell FA, and hence I corrected for two tests. The same corrections were applied for tests on CVs of traits.

For the tests on principal components based on body weight and head width, I calculated the powers as in Fjerdingstad et al. (2003) (using the program G*POWER, Erdfelder et al. 1996) by combining the powers of individual tests, but taking into account the corrections for multiple tests (i.e. the reductions of the significance level). I examined the powers of my tests to detect associations, explaining 9, 16, and 25% of the variation in general size (i.e. correlations of r=0.30, 0.40, and 0.50, respectively). A quantification of the overall power of all the tests that I performed (i.e. including the tests on energy stores, wing traits and sperm content, and FA) was not performed. Such a quantification would be complex because some of the additional traits measured are inter-correlated at the colony level, to some degree, with body weight.

Results

All morphological traits

All traits showed considerable variation among individuals in the population samples examined (Table 1). The levels of within-individual variation (fluctuating asymmetry, FA) were, however, low for two of the three traits examined (wing length and width in males, Table 1).

Body weight and head width

Overall mean size ($PC1_{mean}$) was not positively associated with the effective number of patrilines per colony in any analysis (Table 2). In 1998, however, colonies with a high production of sexuals produced males that were significantly larger overall (Table 2). Also, in 1997, colony means of body weight relative to head width ($PC2_{mean}$) in young queens was positively associated with a high sexual production (standardised regression coefficient β=0.50, df=18, t=2.4, P=0.02). In this year (1997), moreover, such females were produced with a significantly higher frequency by colonies with high intracolonial genetic diversity (more than one patriline) (β=0.48, df=18, t=2.5, P=0.02). These findings remained significant after correction for multiple tests. No other significant associations involving $PC2_{mean}$ were found (results not shown).

No significant association between variation in sexual size ($PC1_{CV}$ based on colony trait CVs) and the effective number of patrilines per colony was found (Table 3). The only significant relationship involving $PC1_{CV}$ was that of colonies that produced many sexuals tending to produce females with a lower colony CV for body weight in 1998 (Table 3). This association did not, however, remain significant when correcting for multiple tests. No significant associations were found for $PC2_{CV}$ (results not shown).

Table 1 Population coefficients of variation of phenotypic traits among individuals (in %) and FA of wing traits in *Lasius niger*. Five males and seven young full-sister queens were examined per colony. The numbers of single- and multiple-paternity colonies examined are given in parentheses (all analyses presented in the text and tables are, however, based on the effective number of patrilines per colony)

	Males-1997	Males-1998	Queens-1997	Queens-1998
	(9/12)	(10/14)-but (12/15) for % sugar test	(7/15)	(11/20)-but (8/18) for % fat tests
Body weight	14.5	19.0	6.7	13.0
Head width	3.3	3.5	2.5	4.3
Sperm load	61.0	-	-	-
Tarsal length	4.8	-	-	-
Wing length	4.1	-	-	-
Wing width	4.3	-	-	-
% sugar	-	41.1	-	-
% fat	-	-	-	16.6
FA wing length in %	0.5			
FA wing width in %	0.7			
FA wing cell in %			2.7	

Table 2 Relationships of general size ($PC1_{mean}$ of colony mean body weight and head width) of individual sexuals with the effective number of patrilines per colony, the production of sexuals, and the proportional investment in females by colonies. Given are standardised partial regression coefficients. Values significant after correction for multiple tests given in **bold**. $PC1_{mean}$ explained 72–82% of the variation and was positively associated with both original variables (loadings 0.85–0.90; maximum possible value: 1.0)

	Males 1997	Males 1998	Queens 1997	Queens 1998
Effective no. of patrilines	0.25	–0.11	0.28	0.06
Production of sexuals	0.15	**0.57*****	0.19	0.17
Prop. investment in females	–0.06	–0.04	–0.24	0.31

* and *** indicate P<0.05 and P<0.005, respectively (see Tables 3, 5, 6).

Table 3 Relationships of variation in size ($PC1_{CV}$ of colony CVs of body weight and head width) of individual sexuals with the effective number of patrilines per colony, the production of sexuals and the proportional investment in females by colonies. All notations as in Table 2. $PC1_{CV}$ explained 50–61% of the variation and was positively associated with both original variables (loadings: 0.71–0.78)

	Males 1997	Males 1998	Queens 1997	Queens 1998
Effective no. of patrilines	0.06	–0.17	0.23	0.22
Production of sexuals	–0.13	0.15	–0.21	–0.39*
Prop. investment in females	–0.20	0.20	–0.40	0.08

Table 4 Associations of general trait size ("Mean") ($PC1_{add.mean}$ of colony mean sperm production, tarsal length, wing length and width), and of variation in trait size ("CV") ($PC1_{add.CV}$ of colony CVs of the same traits), with the effective number of patrilines per colony, the production of sexuals, and the investment in females by colonies. Notations as in Table 2. $PC1_{add.mean}$ explained 67% of the variation and was positively associated with all original variables (loadings: 0.57–0.95). $PC1_{add.CV}$ explained 44% of the variation in colony CVs of the original traits and was positively associated with all these traits (loadings 0.12–0.93)

	Males 1997	
	Mean	CV
Effective no. of patrilines	0.42	–0.07
Production of sexuals	0.23	–0.17
Prop. investment in females	–0.10	–0.24

Table 5 Relationships of direct colony means and CVs of % carbohydrates in males and % fat in young queens with the effective number of patrilines per colony, colony production of sexuals and proportional investment in females. All notations as in Table 2

	Males 1998		Queens 1998	
	Mean	CV	Mean	CV
Effective no. of patrilines	–0.27	0.18	–0.12	–0.03
Production of sexuals	0.38*	0.13	0.31	–0.36
Prop. investment in females	–0.14	0.09	0.40*	–0.27

Sperm load, tarsal lengths, forewing length and width in males

General size ($PC1_{add.mean}$, based on colony mean trait values) showed no significant associations with the effective number of patrilines per colony, colony productivity or sex ratio (Table 4). Also, no significant associations were found for the second or third principal components (results not shown) (PC2 and PC3 constituted various contrasts between the different original variables). Similarly, for the PCs based on colony trait CVs no significant associations were found (Table 4).

Energy stores: carbohydrate content of males and fat content of young queens

The mean carbohydrate content of males (in % of body weight) was not related to the effective number of patrilines per colony or the investment in females by colonies (Table 5). Variation in carbohydrate content (CV) also showed no significant associations with these potential explanatory variables (Table 5). Males from colonies that invested much in the production of sexuals did, however, have a higher carbohydrate content (Table 5), but this did not remain significant when correcting for multiple tests. The mean fat content of queens (in % of body weight) was positively associated with the proportional investment in females by colonies, but this association did not hold after corrections for multiple tests. There were no significant associations between mean fat content of queens and the effective number of patrilines per colony or total production of sexuals, or between variation in fat content (CV) and the effective number of patrilines, productivity or colony investment in females (Table 5).

Fluctuating asymmetry

Colony mean FA of males was not significantly related to the effective number of patrilines per colony, sexual production or sex ratio of colonies (Table 6). In young queens, FA was also not related to the effective number of patrilines per colony. However, colony mean FA of young queens was very close to being significantly positively associated (P=0.06) with the sexual biomass produced by colonies in 1997 (but not in 1998) (Table 6). Also, FA of young queens in 1997 showed a significant negative association with the investment in females by colonies (Table 6). This result did, however, turn borderline non-significant after correction for multiple tests.

Power analyses

My power analyses showed that associations in which the effective number of patrilines explained 9, 16 and 25% of the variation in mean general size (PC1 based on estimated body weight and head width) should have been detected with a power of 61%, 86% and 98%, respectively. My power to detect associations between the effective number of patrilines and the coefficients of variation of size was the same.

Discussion

Little support was found in the present study for the hypothesis that increased intra-colonial genetic diversity enhances colony homeostasis, and thereby reduces developmental stress in social insects. *L. niger* ant sexuals

Table 6 Relationships of colony mean fluctuating asymmetry (*FA*) of wing traits and the number of patrilines per colony, the production of sexuals, and the proportional investment in females by colonies. All notations as in Table 2

	Males 1997	Queens 1997	Queens 1998
Effective no. of patrilines	–0.31	0.08	–0.03
Production of sexuals	–0.38	0.45	0.06
Prop. investment in females	0.21	–0.18	–0.41*

from single-paternity colonies were not of a smaller size and neither were the colony coefficients of variation or the levels of fluctuating asymmetry greater in such colonies, in contrast to my predictions that such low-diversity colonies should constitute more stressful colony environments than should multiple-paternity colonies. In fact, there were no certain significant relationships between overall sexual size, variance in overall sexual size, or fluctuating asymmetry of morphological traits and intra-colonial genetic diversity.

There was only one association that indicated an effect of increased intra-colonial genetic diversity. Body weight relative to head width in young queens was significantly positively related to the number of fathers per colony in 1 out of 2 years, and this significance held after correcting for multiple tests. This finding is, however, not necessarily an indication that young queens from multiple-paternity colonies are likely to have a higher personal fitness than queens from single-paternity colonies (large queens have a greater reproductive performance in *L. niger*, Fjerdingstad and Keller 2004), because the overall size (this study) and body weight (results not shown) of young queens was not higher in multiple-paternity colonies. Also, relative fat content, which is likely very important for queen survival during the claustral phase of colony foundation (cf. Keller and Passera 1989) (queens rear their first brood on their body reserves in *L. niger*), showed no significant association with the levels of intra-colonial genetic diversity.

The scarcity of evidence that intra-colonial genetic diversity affects the means, variances and FA of sexual phenotype in *L. niger* seems unlikely to be due to all the examined traits being under strong developmental control (strongly canalized) (Palmer 1994a; Clarke 1998; Woods et al. 1999) and hence poor indicators for environmental and developmental stress. The colony coefficients of variation (Table 1) for the traits used in my study were of the same level as the CVs reported for various morphological traits in *Drosophila* flies that did respond to environmental stress (Imasheva et al. 1997; Hoffmann and Schiffer 1998; Woods et al. 1999; see also Clarke 1998), and some of the traits examined in my study even had very large CVs (sperm load, sugar content and fat content). Hence, the great majority of the traits I used as indicators for colony stress levels showed no evidence of strong canalization. Also, I examined a great variety of traits, which should counter the potential problem of inter-trait variation in responsiveness to stress (Clarke 1998; Woods et al. 1999).

My indicator traits for within-individual variation in males were, however, likely less good. The fluctuating asymmetry of the two traits measured in males (wing length and width) were significantly inter-correlated, suggesting that wing FA constituted a good indicator of the developmental stability of males (cf. Palmer 1994a). The absolute FA for these traits was, however, quite low (only 0.5–0.7% of trait size), which may indicate that wing dimensions are strongly canalized (cf. Stearns and Kawecki 1994). A strong canalisation would mean that wing asymmetry would only be little affected by environmental conditions and, hence, not constitute a good indicator trait for a stress analysis such as mine.

The scarcity of support for the hypothesis that multiple paternity enhances the developmental homeostasis of *L. niger* sexuals in my study is not due to poor powers of test. I would have detected associations in which the number of fathers explained 9, 16, and 25% of the variation in mean general size (as estimated by body weight and head width) with a power of 61%, 86% and 98%, respectively. I had the same power of test to detect associations between the number of fathers and the coefficients of variation of size. The actual power of all of my tests is still higher because I performed tests on additional variables (sperm load, tarsal length, wing length and width, sugar content, fat content and FAs). It cannot be excluded that high intra-colonial genetic diversity may slightly increase the ability of workers to maintain a stable colony environment and so lead to a slightly greater developmental homeostasis for sexuals, such a small effect being very difficult to detect. If the benefits of increased intra-colonial genetic diversity for colony homeostasis and the developmental homeostasis of sexuals are quite small then this, however, also implies that homeostasis-based selection favouring multiple paternity should at best be modest. My study cannot, however, exclude that in exceptionally harsh years a high genetic diversity might bring benefits to *L. niger* colonies.

In contrast to the weak evidence for any impact of intra-colonial genetic diversity on phenotypic variation in sexuals of *L. niger*, I found several pieces of support for my hypothesis that such variation may be associated with the total reproductive output, and tentative support that it may be related to the preferred sex ratio of colonies. Male size was significantly larger the greater the total reproductive output of colonies in 1998, and young queens from more productive colonies had a higher body weight relative to their head width. Also, the flight-fuel stores (carbohydrate content) of males in 1997 and the fluctuating asymmetry of wing cells in young queens tended to be greater the higher the reproductive effort of colonies in 1 year (though these associations were not significant after correcting for multiple tests). The greater body weight or greater body weight relative to head width found in sexuals from colonies with a high reproductive output is consistent with the hypothesis by Rosenheim et al. (1996) that colonies would invest more in individual sexuals when resources become abundant (see also Karsai and Hunt 2002; Ode and Rissing 2002). However, colonies should of course only do so if investing more in individual sexuals will not decrease the fitness of these sexuals. For *L. niger* queens, a high body weight was positively associated with fecundity in 1997 and 1998, and queens of medium size had the highest across-year survival probability (Fjerdingstad and Keller 2004). For males, however, no data are available on individual-level fitness in relation to the amount of resources invested in them for 1998 and so it cannot be stated conclusively here that an increased investment in individual males by very

productive colonies was adaptive. Also, in 1998, queens from colonies with a high reproductive output tended (though not significant after correction for multiple tests) to have a higher fluctuating asymmetry of their wing cells, suggesting that such colonies might constitute more stressful instead of more beneficial environments for developing sexuals.

As hypothesized, the sex ratio of colonies in several cases showed a tendency to be associated with the variance in sexual phenotype across and within individuals. The size of the fat stores with which young queens were endowed in 1998 tended to be greater the more of its resources their natal colony had invested in producing young queens (relative to its investment in males). Also, the fluctuating asymmetry of wing cells in queens tended to be lower the more the sex ratio of their natal colony was female-biased. These findings are consistent with my hypothesis that sexuals may be treated better in colonies in which they belong to the majority sex. This pattern is not a consequence of worker sex-ratio preferences varying in response to the mating frequency of their mother queen (cf. split-sex ratios; Ratnieks and Boomsma 1995; Sundström et al. 1996) because in *L. niger* such adaptive sex-ratio biasing does not occur (Fjerdingstad et al. 2002). The association of the state of young queens and the sex ratio of their natal colonies may instead result from sexuals of different genders having different resource or micro-environmental requirements and so suffering from being reared under the regime used for rearing sexuals of the opposite gender.

In conclusion, I found little evidence that increased intra-colonial genetic diversity enhances the developmental homeostasis of sexuals in *L. niger*, whereas colony productivity was found to be associated in some samples with the phenotypic variation of sexuals. Also, I found a tendency for associations between colony sex-ratio preferences and sexual phenotype. My finding little support for increased intra-colonial genetic diversity having any effect on estimators of colony environment is consistent with previous work by myself and collaborators on *L. niger* showing that multiple-paternity colonies did not exhibit a better reproductive performance on average, and did not have any certain survival advantage compared to single-paternity colonies (Fjerdingstad et al. 2003). Lack of benefits of increased genetic diversity have also been found in several other studies (Page et al. 1995; Fuchs et al. 1996; Kraus and Page 1998; Neumann and Moritz 2000; review by Crozier and Fjerdingstad 2001) though support for the hypothesis that high intra-colonial genetic diversity may increase colony performance has been found for honeybees (in some studies), *Bombus terrestris* bumble bees, and *Pogonomyrmex* harvester ants (e.g. Schmid-Hempel 1998; Cole and Wiernasz 1999; Baer and Schmid-Hempel 2001—but see Fjerdingstad and Keller 2000; Oldroyd et al. 1992; Fuchs and Schade 1994; Crozier and Fjerdingstad 2001; Tarpy 2003) (see also Bourke and Franks 1995; Boomsma and Ratnieks 1996; Crozier and Pamilo 1996).

The causes for this variation in finding support or lack of support for benefits of high intra-colonial genetic diversity in social insects have not yet been established (Fjerdingstad et al. 2003; see also Kraus and Page 1998; Sherman et al. 1998), but genuine differences among different species seem to exist (Fjerdingstad et al. 2003). It is possible that multiple mating by queens is governed by different selective factors in species with a moderate number of mates per queen (as, for example, *L. niger*) than in species with very many mates per queen (e.g. honey bees, *Pogonomyrmex* ants). Tests for selective reporting and publication bias (cf. Palmer 1994b and references therein) will, however, be required for a thorough evaluation of the insight that existing publications give into the genuine relationships between intra-colonial genetic diversity and colony performance in social insects.

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References

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Anim Behav 60:145–164

Aron S, Vargo EL, Passera L (1995) Primary and secondary sex ratios in monogyne colonies of the fire ant. Anim Behav 49:749–757

Backus VL (1993) Packaging of offspring by nests of the ant *Leptothorax longispinosus*: parent-offspring conflict and queen-worker conflict. Oecologia 95:283–289

Baer B, Schmid-Hempel P (2001) Unexpected consequences of polyandry for parasitism and fitness in the bumblebee *Bombus terrestris*. Evolution 55:1639–1643

Boomsma JJ, Grafen A (1991) Colony-level sex ratio selection in the eusocial Hymenoptera. J Evol Biol 4:383–407

Boomsma JJ, Isaaks JA (1985) Energy investment and respiration in queens and males of *Lasius niger* (Hymenoptera: Formicidae). Behav Ecol Sociobiol 18:19–27

Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. Philos Trans R Soc Lond B 351:947–975

Boomsma JJ, Van der Have TM (1998) Queen mating and paternity variation in the ant *Lasius niger*. Mol Ecol 7:1709–1718

Bourke AFG, Franks NR (1995) Social evolution in ants. Princeton University Press, New Jersey

Chapuisat M, Keller L (1999) Testing kin selection with sex-allocation data in eusocial Hymenoptera. Heredity 82:473–478

Clarke GM (1998) The genetic basis of developmental stability. V. Inter- and intra-individual variation. Heredity 80:562–567

Cole BJ, Wiernasz DC (1999) The selective advantage of low relatedness. Science 285:891–893

Cook JM (1993) Sex determination in Hymenoptera: a review of models and evidence. Heredity 71:421–435

Crozier RH, Fjerdingstad EJ (2001) Polyandry in social Hymenoptera—disunity in diversity? Ann Zool Fenn 38:267–285

Crozier RH, Page RE (1985) On being the right size: male contributions and multiple mating in social Hymenoptera. Behav Ecol Sociobiol 18:105–115

Crozier RH, Pamilo P (1996) Evolution of social insect colonies—sex allocation and kin selection. Oxford University Press, Oxford

Erdfelder E, Faul F, Buchner A (1996) GPOWER: a general power analysis program. Behav Res Methods Instrum Comp 28:1–11. http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/index.html

Fjerdingstad EJ, Boomsma JJ (1997) Variation in size and sperm content of sexuals in the leafcutter ant *Atta colombica*. Insectes Soc 44:209–218

Fjerdingstad EJ, Keller L (2000) The nature of ant colony success. (Technical comment). Science http://www.sciencemag.org/cgi/content/full/287/5457/1363b

Fjerdingstad EJ, Keller L (2004) Relationships between phenotype, mating behaviour, and fitness of queens in the ant *Lasius niger*. Evolution (in press)

Fjerdingstad EJ, Gertsch PJ, Keller L (2002) Why do some social insect queens mate with several males?—testing the sex ratio manipulation hypothesis in *Lasius niger*. Evolution 56:553–562

Fjerdingstad EJ, Gertsch PJ, Keller L (2003) The relationship between multiple mating by queens, within-colony genetic variability and fitness in the ant *Lasius niger*. J Evol Biol 16:844–853

Fuchs S, Schade V (1994) Lower performance in honeybee colonies of uniform paternity. Apidologie 25:155–168

Fuchs S, Büchler R, Hoffmann S, Bienefeld K (1996) Non-additive colony performances by inseminating queens with mixed sperm of several carniolan breeder lines. Apidologie 27:304–306

Hamilton WD (1964) The genetical evolution of social behaviour. I, II. J Theor Biol 7:1–52

Hamilton WD (1987) Kinship, recognition, disease, and intelligence: constraints of social evolution. In: Itô Y, Brown JL, Kikkawa J (eds) Animal societies: theories and facts. Japan Sci Soc, Tokyo, pp 81–102

Henshaw MT, Queller DC, Strassmann JE (2002) Control of male production in the swarm-founding wasp, *Polybioides tabidus*. J Evol Biol 15:262–268

Herbers JM (1990) Reproductive investment and allocation ratios for the ant *Leptothorax longispinosus*: sorting out the variation. Am Nat 136:178–208

Hoffmann AA, Schiffer M (1998) Changes in the heritability of five morphological traits under combined environmental stresses in *Drosophila melanogaster*. Evolution 52:1207–1212

Hoffmann AA, Woods R (2001) Trait variability and stress: canalisation, developmental stability and the need for a broad approach. Ecol Lett 4:97–101

Hogg ID, Eadie JM, Williams DD, Turner D (2001) Evaluating fluctuating asymmetry in a stream-dwelling insect as an indicator of low-thermal stress: a large-scale field experiment. J Appl Ecol 38:1326–1339

Hölldobler B, Wilson EO (1990) The ants. Springer, Berlin Heidelberg New York

Imasheva AG, Loeschke V, Zhivotovsky LA, Labezny OE (1997) Effects of extreme temperatures on phenotypic variation and developmental stability in *Drosophila melanogaster* and *Drosophila buzzati*. Biol J Linn Soc 61:117–126

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biol Rev 75:21–64

Jobson JD (1996) Applied multivariate data analysis: categorical and multivariate methods. Springer, Berlin Heidelberg New York

Karsai I, Hunt JH (2002) Food quantity affects traits of offspring in the paper wasp *Polistes metricus*. Environ Entomol 31:99–106

Keller L, Passera L (1989) Size and fat content of gynes in relation with the mode of colony founding in ants (Hymenoptera: Formicidae). Oecologia 80:236–240

Keller L, Reeve HK (1994) Genetic variability, queen number, and polyandry in social Hymenoptera. Evolution 48:694–704

Keller L, Reeve HK (1995) Why do females mate with multiple males? The sexually selected sperm hypothesis. Adv Stud Anim Behav 24:291–315

Keller L, Aron S, Passera L (1996) Internest sex-ratio variation and male brood survival in the ant *Pheidole pallidula*. Behav Ecol 7:292–298

Kraus B, Page RE (1998) Parasites, pathogens and polyandry in social insects. Am Nat 151:383–391

Leung B, Forbes MR (1996) Fluctuating asymmetry in relation to stress and fitness: effects of trait type as revealed by meta-analysis. Ecoscience 3:400–413

Mehdiabadi NJ, Reeve HK, Mueller UG (2003) Queens versus workers: sex ratio conflict in eusocial Hymenoptera. Trends Evol Ecol 18:88–93

Møller AP, Swaddle JP (1998) Asymmetry, developmental stability, and evolution. Oxford University Press, Oxford

Neumann P, Moritz RFA (2000) Testing genetic variance hypotheses for the evolution of polyandry in the honeybee (*Apis mellifera* L.). Insectes Soc 47:271–279

Ode PJ, Rissing SW (2002) Resource abundance and sex allocation by queen and workers in the harvester ant, *Messor pergandei*. Behav Ecol Sociobiol 51:548–556

Oldroyd BP, Rinderer TE, Harbo JR, Buco SM (1992) Effects of intracolonial genetic diversity on honey bee (Hymenoptera: Apidae) colony performance. Ann Entomol Soc Am 85:335–343

Page RE, Robinson GE, Fondrk MK, Nasr ME (1995) Effects of worker genotypic diversity on honey bee colony development and behavior (*Apis mellifera* L.). Behav Ecol Sociobiol 36:387–396

Palmer AR (1994a) Fluctuating asymmetry analyses: a primer. In: Markow T (ed) Developmental instability: its origins and evolutionary implications. Kluwer, Dordrecht, pp 335–364

Palmer AR (1994b) Detecting publication bias in meta-analyses: a case study of fluctuating asymmetry and sexual selection. Am Nat 154:220–233

Palmer AR, Strobeck C (1986) Fluctuating asymmetry: measurement, analyses and patterns. Annu Rev Ecol Syst 17:391–421

Palmer KA, Oldroyd B (2000) Evolution of multiple mating in the genus *Apis*. Apidologie 31:235–248

Passera L, Aron S (1996) Early sex discrimination and male brood elimination by workers of the Argentine ant. Proc R Soc Lond B 263:1041–1046

Passera L, Keller L, Grimal L, Chautems D, Cherix D, Fletcher DJC, Fortelius W, Rosengren R, Vargo EI (1990) Carbohydrates as energy-source during the flight of sexuals of the ant *Formica lugubris* (Hymenoptera, Formicidae). Entomol Gen 15:25–32

Polak M, Opoka, R, Cartwright IL (2002) Response of fluctuating asymmetry to arsenic toxicity: support for the developmental selection hypothesis. Environ Pollut 118:19–28

Queller DC, Strassmann JE (1998) Kin selection and social insects. Bioscience 48:165–175

Ratnieks FLW, Boomsma JJ (1995) Facultative sex allocation by workers and the evolution of polyandry by queens in social Hymenoptera. Am Nat 145:969–993

Reichardt AK, Wheeler DC (1995) Estimation of sperm numbers in insects by fluometry. Insectes Soc 42:449–452

Robinson GE, Page RE (1989) Genetic basis for division of labor in an insect society. In: Breed MD, Page RE (eds) The genetics of social evolution. Westview, Boulder, Colo, pp 61–80

Rosenheim JA, Nonacs P, Mangel M (1996) Sex ratios and multifaceted parental investment. Am Nat 148:501–535

Sakaluk SK, O'Day DH (1984) Hoechst staining and quantification of sperm in the spermatophore and spermatheca of the decorated cricket, *Gryllodes supplicans* (Orthoptera: Gryllidae). Can Entomol 116:1585–1589

Schmid-Hempel P (1998) Parasites in social insects. Princeton University Press, Princeton, NJ

Sherman PW, Seeley TD, Reeve HK (1988) Parasites, pathogens, and polyandry in social Hymenoptera. Am Nat 131:602–610

Sherman PW, Seeley TD, Reeve HK (1998) Parasites, pathogens, and polyandry in honey bees. Am Nat 151:392–396

Simmons LW (2001) Sperm competition and its evolutionary consequences in the insects. Princeton University Press, Princeton

Stearns SC, Kawecki TJ (1994) Fitness sensitivity and the canalization of life-history traits. Evolution 48:1438–1450

Sundström L, Chapuisat M, Keller L (1996) Conditional manipulation of sex ratios by ant workers: a test of kin selection theory. Science 274:993–995

Tarpy DR (2003) Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. Proc R Soc Lond B 270:99–103

Van der Have TM, Boomsma JJ, Menken SBJ (1988) Sex investment ratios and relatedness in the monogynous ant *Lasius niger* (L.). Evolution 42:160–172

Van Handel E (1985) Rapid determination of glycogen and sugars in mosquitoes. J Am Mosq Contr Assoc 1:299–304

Van Handel E, Day JF (1988) Assay of lipids, glycogen, and sugars in individual mosquitoes: correlation with wing-length in field-collected *Aedes vexans*. J Am Mosq Contr Assoc 4:549–550

Ward PA (1983) Genetic relatedness and colony organization in a species complex of ponerine ants. II. Patterns of sex ratio investment. Behav Ecol Sociobiol 12:301–307

Wiernasz DC, Slater AK, Abell AJ, Cole BJ (2001) Male size, sperm transfer, and colony fitness in the western harvester ant, *Pogonomyrmex occidentalis.* Evolution 55:324–329

Woods RE, Sgro CM, Hercus MJ, Hoffmann AA (1999) The association between fluctuating asymmetry, trait variability, trait heritability, and stress: a multiply replicated experiment on combined stresses in *Drosophila melanogaster.* Evolution 53:493–505